MATERIALS AND METHODS FOR RAPID AND SPECIFIC DETECTION OF COCAINE

CROSS-REFERENCE TO A RELATED APPLICATION

This application claims the benefit of U.S. Provisional Application Ser. No. 62/073,718, filed Oct. 31, 2014, which is incorporated herein by reference in its entirety.

GOVERNMENT SUPPORT

The subject invention was made with government support under a research project supported by National Institute of Justice under Grant No. 2013-DN-BX-K032. The government has certain rights in this invention.

The Sequence Listing for this application is labeled "SeqList-11Jan16-ST25.txt", which was created on Jan. 11, 2016, and is 8 KB. The entire content is incorporated herein by reference in its entirety.

BACKGROUND OF INVENTION

Cocaine is a central nervous system stimulant that increases levels of dopamine and potently inhibits neurotransmitter reuptake at the synapse. Abuse of cocaine has been shown to cause anxiety, paranoia, mood disturbances, organ damage, and violent behavior. Therefore, rapid detection of cocaine is needed to confirm suspicion of recent use in impaired driver investigations or to assist in overdose treatment in medical emergency settings.

Various immunoassays have been developed for the detection of cocaine and/or its major metabolite benzoylecgonine in biofluids, including the enzyme-linked immunosorbent assay (ELISA) and the EMIT II Plus Cocaine Metabolite Assay. Unfortunately, the use of these assays is often limited because of the high cost of generating antibodies and issues with poor specificity. These antibody-based tests often cannot distinguish between the targeted drug and structurally similar substances, resulting in cross reactivity-related false positives.

Aptamers are single-stranded RNA or DNA molecules selected in vitro via Systematic Evolution of Ligands by Exponential Enrichment (SELEX) (Tuerk, C.; Gold, L. *Science.* 1990, 249, 505-510) to specifically bind to targets with high affinity, and they offer a practical alternative to antibodies for the detection of nucleic acids, proteins and small molecules. Compared to antibodies, aptamers are relatively fast and cheap to produce, and can be chemically synthesized with extreme accuracy and reproducibility. In aptamers having a three-way junction structure the intact stem 3 is essential for cocaine binding, while stem 1 and stem 2 both contribute to the stability of the target-induced three-way junction structure (D. Roncancio, H. Yu, X. Xu, S. Wu, R. Liu, J. Debord, X. Lou, Y. Xiao, *Anal. Chem.* 2014, 86, 11100-6). Due to the high stability of DNA aptamers, they can be stored and used under harsher conditions, and can achieve a longer shelf life (W. Mok, Y. Li, *Sensors* 2008, 8, 7050-7084). It is possible to generate unstructured aptamers that form specific secondary structures such as three-way junctions (M. N. Stojanovic, P. de Prada, D. W. Landry, *J. Am. Chem. Soc.* 2001, 123, 4928-31; K.-A. Yang, M. Barbu, M. Halim, P. Pallavi, B. Kim, D. M. Kolpashchikov, S. Pecic, S. Taylor, T. S. Worgall, M. N. Stojanovic, Nat. Chem. 2014, 6, 1003-8) or G-quadruplexes (L. C. Bock, L. C. Griffin, J. A. Latham, E. H. Vermaas, J. J. Toole, Nature 1992, 355, 564-6; D. E. Huizenga, J. W. Szostak, Biochemistry 1995, 34, 656-665) upon target binding. Such target-induced conformational changes can be readily exploited for specific target detection in a variety of applications including medical diagnostics, environment monitoring and drug screening (T. Mairal, V. C. Ozalp, P. Lozano Sánchez, M. Mir, I. Katakis, C. K. O'Sullivan, Anal. Bioanal. Chem. 2008, 390, 989-1007; J. H. Lee, M. V Yigit, D. Mazumdar, Y. Lu, Adv. Drug Deliv. Rev. 2010, 62, 592-605; E. J. Cho, J.-W. Lee, A. D. Ellington, Annu. Rev. Anal. Chem. (Palo Alto. Calif.). 2009, 2, 241-64). Aptamer-based sensors have gained popularity because of their simplicity and specificity. For example, derivatives of the MNS-4.1 cocaine-binding aptamer (Stojanovic, M. N.; Prada, P.; Landry, D. W. *J. Am. Chem. Soc.* 2000, 122, 11547-11548) have been labeled with sensing elements such as fluorophore/quencher pairs (Stojanovic, M. N.; Prada, P.; Landry, D. W. *J. Am. Chem. Soc.* 2001, 123, 4928-4931); magnetic or metallic nanoparticles (Du, Y.; Li, B.; Guo, S.; Zhou, Z.; Zhou, M.; Wang, E.; Dong, S. *Analyst* 2011, 136, 493-497; Zhang, J.; Wang, L.; Pan, D.; Song, S.; Boey, F. Y. C.; Zhang, H.; Fan, C. *Small* 2008, 4, 1196-1200; Liu, J.; Lu, Y. *Angew. Chem. Int. Ed* 2006, 45, 90-94), quantum dots (Zhang, C. Y.; Johnson, L. W. *Anal. Chem.* 2009, 81, 3051-3055; Liu, J.; Lee, J. H.; Lu, Y. *Anal. Chem.* 2007, 79, 4120-4125) and methylene blue (Baker, B. R.; Lai, R. Y.; Wood, M. S.; Doctor, E. H.; Heeger, A. J.; Plaxco, K. W. *J. Am. Chem. Soc.* 2006, 128, 3138-3139; Swensen, J. S.; Xiao, Y.; Ferguson, B. S.; Lubin, A. A.; Lai, R. Y.; Heeger, A. J.; Plaxco, K. W.; Soh, H. T. *J. Am. Chem. Soc.* 2009, 131, 4262-4266) to achieve specific detection of cocaine.

In the absence of cocaine, the aptamer population exists in an equilibrium state consisting of both folded and unfolded structures (Neves, M. A.; Reinstein, O.; Johnson, P. E. *Biochemistry* 2010, 49, 8478-8487), where the folded structures generate a background signal. When challenged with cocaine, the unfolded aptamers undergo a target-induced conformational change and form a non-canonical three-way junction that binds cocaine, producing a signal change. This limited target-induced fluorescence change results in a high detection limit (10 μM) even under optimal conditions, and the reason may be due to inefficient proximity quenching, low aptamer target binding affinity, or both (Stojanovic, M. N.; Prada, P.; Landry, D. W. *J. Am. Chem. Soc.* 2001, 123, 4928-4931). In addition, target-induced conformational changes are hard to control, especially for small-molecule-binding aptamers that have relatively high (~μM) dissociation constants (KD) (M. McKeague, M. C. Derosa, J. Nucleic Acids 2012, 2012, DOI 10.1155/2012/748913).

Different strategies such as target-displacement have been used to increase the sensitivity of aptamer-based detection. For example, Stojanovic's group used unmodified MNS-4.1 (FIG. **1**A, MNS-4.1) to construct a colorimetric cocaine sensor based on cocaine-mediated displacement of a cyanine dye (diethylthiotricarbocyanine iodide; Cy7) from the dye-aptamer complex (Stojanovic, M. N.; Landry, D. W. *J. Am. Chem. Soc.* 2002, 124, 9678-9679). They observed decreased absorbance of Cy7 at 760 nm with increasing cocaine concentrations in the range of 2 to 600 μM and increased sensitivity compared to the corresponding fluorescence sensor due to the high binding affinity of unmodified MNS-4.1 aptamer for cocaine. However, the MNS-4.1 aptamer formed a three-way junction even before binding cocaine (M. N. Stojanovic, D. W. Landry, J. Am. Chem. Soc. 2002, 124, 9678-9) leading to high background signal. In order to achieve a target-induced conformational change, Stojanovic et al. had truncated the sequence to destabilize